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Effects of the Food Quality on the Appearance of Yellowtail Streptococcicosis*

Michiko TANIGUCHI**

To clarify the effects of food quality on the appearance of yellowtail streptococcicosis, the tolerance of the fish fed with various quality of foods during the experimental period was examined based on the artificial peroral inoculation method. The vitamin mixture was surely effective to decrease mortality from the diseases during hot season. When garlic extract was fed besides the vitamin mixture, seriously infected individuals were small in number. The incidence of the disease was very low in the experimental group fed with frozen fish in comparison with the experimental group fed with thawed fish. The experimental groups fed with washed fish or the thawed fish mixed with ethoxyquin also showed low incidence of the diseases compared with the group fed with thawed fish. Conclusively, feeding with frozen fish before thawing was the best among the other groups fed with thawed fish.

It is important with regard to yellowtail streptococcicosis to know the relationship between the feed conditions and the occurrence of the disease. We showed in our former reports[1,2] that to know such relationship it is useful to inject the pathogenic bacteria under the skin of the feed fish which will be ingested by the yellowtail. We demonstrated in those reports that the form and types of feed used for bacterial administration affect greatly the occurrence of the disease

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and suggested that the feed used during the period preceding the bacterial administration could also have an important effect upon the occurrence of the disease. The question as to whether or not the feed used for rearing affects the occurrence does not only regard the examination of the testing method. It is also important for the measures taken to prevent epidemics. It has already been pointed out in the results of the already performed epidemiological surveys[3] that the type of feed used, the use or no use of nutriments, the methods of thawing the feed and the like have a close relationship with the rate of injury. One has also noted at the actual aquaculture stations that harm from streptococcicosis tends to be great when much harm is done through feed-related disease. Often numerous fish affected by streptococcicosis suffer also from feed-related conditions. It has often been possible to alleviate and prevent the present disease through determined use of combined vitamins and the like.

We will try in the present study to clarify experimentally the correlation between the feed used for rearing and the occurrence of disease, using the artificial oral inoculation method[1,2].

Testing method

Sample fish

Like in our former study[1] we used cultured yellowtail Seriola quinqueradiata. For the rearing feed, we minced unthawed frozen sand launce and added combined vitamins. We took from among the fish reared in this fashion the required numbers of individuals, placed them in

separate netted enclosures or concrete tank and gave them for a given period of time determined particular types of feed. Thereafter, we used them for infection tests. We will call this period the rearing period and the feed used during that time the rearing feed. In the tests performed to know the effect of nutriment addition, we used various feeds and compared the rates of disease occurrence, establishing a nutriment-addition group and another group without nutriment addition. With regard to the nutriments, we admixed spreader agents to combined vitamins (Hamachiedo S from the Takeda Yakuhin Kogyo Co.) and used this at the rate of 0.1% vs. the fish body weight. Thereafter we established separate groups given ursodeoxycholic acid (urso 20 from the Tokyo Tanabe Seiyaku Co.), garlic extract (Bitarik* U from the Fuji Sangyo Co.) and ferment calcium lactate (CV calcium from the Shimahisa Yakuhin Co.) together with the combined vitamins. We compared with the group not given any addition. We used as feed thawed frozen sardine. We gave deoxycholic acid to the rate of 0.04% vs. fish body weight. The rate for garlic extract and ferment calcium lactate was 0.1%.

In the test conducted to know the correlation with the thawing method, we established a thaw and a no-thaw group. In part of the tests we added a group for which water washing was performed after the thaw and a group for which ethoxyquine was added following the thaw. To thaw the food we left it for 1 night inside an egg incubator at 23°C. For the feed of the no-thaw group, we minced the feed frozen and kept it at low temperature until the time preceding immediately the

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*Translator's note: Or "Vitarik" or resembling appellation ...

feeding so as to prevent the feed from thawing. As for the feed of the group established for post-thaw washing, we washed with service water feed which had been thawed just as for the thaw group. For the feed of p. 364
the group treated with ethoxyquine addition following feed thawing, we immersed the same thawed feed for 5 min in a 1% ethoxyquine aqueous solution. For the ethoxyquine we used 10% ethoxyzol from the Kōkin Kagaku Co.

Inoculation method and bacterial solution preparation

We followed the method explained in our former report[1].

Bacterial inoculation method

To perform the administration, we injected a bacterial solution under the skin of the feed fish as explained in our former report[1]. However, in order to eliminate the effect of the feed used for bacterial inoculation upon the disease occurrence, we thawed the feed used for bacterial inoculation and minced it to uniformity regardless of the state of the rearing feed.

Determination of disease outbreak

We used the method explained in our former report[1]. In part of the tests however, we proceeded as follows to know the gravity of disease occurrence. We considered as seriously sick those individuals having 3 or more signs of disease in 3 or more places. We observed the proportion of such individuals among all sample fish and used this as severe condition rate. Furthermore, in the detection and examination of the pathogenic bacteria, we considered as individuals showing high-density bacterial detection those individuals in which we

discovered at least 10^4 colonies per 1 g of blood or internal organs. We observed the proportion among all sample fish and used this as high-density bacterial detection rate.

In the compilation of the results, we attached a single test No. (Exp. No.) whenever a test was conducted independently. We attached identical Roman-letter test numbers a, b to test conducted parallelly at the same time.

Results and discussion

Effect of nutriments upon disease outbreak

We tabulated in Table 1 the effect of combined vitamin use during the rearing period upon the occurrence of disease. In Exp. 1, 2 the detection of the pathogen bacterium was difficult because of the appearance of saprophites covering the culture, making a detailed examination impossible. However, with regard to general trends, we noted practically no difference in disease occurrence rate depending on the use or non-use of nutriments. Moreover, in all tests, the death rate was remarkably low in the groups treated with nutriments. Although the rearing period in the present tests was only 10-14 days, one had to note a great difference between the two groups. In Exp. 3, 4 we saw no difference based on the use or non-use of nutriments with regard to disease occurrence rate, bacterial detection rate or death. It seems that the great difference in the effect of nutriment use in Exp. 1, 2 and Exp. 3, 4 is due to a difference in rearing period, that is to say due to the fact of the high-water-temperature period in Exp. 1, 2 and low-water-temperature period in Exp. 3, 4. Although the rearing

period for Exp. 3, 4 was relatively long (30 days), there was no clear effect of the use or non-use of nutriments. This seems not to be unrelated to the fact that the effect of nutritional deficiency in feed is hardly evident at low water temperatures which had been pointed out by Harada[4]. In Exp. 5 we obtained in the group treated with combined vitamins results contrary to those of the other six tests in which the mortality rate was high. The reason seems to be as follows. That is, the feed used for rearing in this test was deteriorated anchovy kept for 2 years in a low-performance storage house. While the drip produced through thawing was left in the feed containers and not ingested by the group not treated with combined vitamins, the drip was almost entirely adsorbed because of the spread effect in the case of the group treated with combined vitamins. So it seems that the bad effect of ingested deteriorated matters was stronger than the effect of the added combined vitamins.

These results seem to indicate that although the effectiveness of the combined vitamins in preventing disease occurrence is determined by the period of usage and the quality of the food, these vitamins must be used constantly as one of the means to lessen the harm from streptococcicosis.

Thereafter, we show in Table 2 the effect of the joint use of combined vitamins and of the diverse nutriments viz. ursodesoxycholic acid, garlic extract, ferment calcium lactate. In the past methods of compiling, almost no difference in disease occurrence rate, bacterial detection rate, death rate was noted between the various groups treated

also with nutriments and the group not treated with the same. If, however, we divide between serious and light disease outbreak we note immediately that the rates of serious illness and of high-density detection are low in all the various groups treated also with nutriments. The values were particularly low in the groups treated with garlic extract. This seems to indicate that this nutriment inhibits the multiplication of pathogens and the occurrence of disease among the fish. Depending on the number of pathogenic bacteria, the strength of the pathogens, the environmental conditions and other disease outbreak factors, it seems that it should be possible to stop the outbreak of the disease.

Effect of the thawing method upon the disease outbreak

Following this, we examined the effect of the method of thawing the frozen feed fish used during the rearing period upon the occurrence of disease. Our results are shown in Table 3. In all the tests, the disease occurrence rate was lower in the no-thaw group than in the groups fed thawed feed. The rate of disease occurrence was slightly lower in the group with washing-by-water performed after thawing and in the ethoxyquine addition group than in the thaw group, that is the group with no treatment performed after thawing. When these 4 types of treating methods were compared, we saw that the disease occurrence rate was particularly low in the no-thaw group. In the present tests we compared by changing only the rearing feed of the host and it seems that the results obtained reflect the resistance of the host. One may say that it has been clarified experimentally that using frozen feed is effective in raising the resistance of the host.

In the relationship between the state of feed used for inoculation and the occurrence of disease explained in our former report, too, it was demonstrated that the rate of disease occurrence was high in the thawed-feed group and that the disease occurrence is enhanced by the multiplication of pathogenic bacteria at the thawing stage. It thus seems that the use of unthawed feed is effective as an epidemiological measure against streptococcicosis as it raises the resistance of the host and as it decreases the risk of oral route contamination through feed. This method of feeding is already used by some aquaculture operators in the Kochi Prefecture, and the prospects seem to be good with regard to epidemic prevention. To promote this method as epidemiological policy, one should pay close attention to the results of the rearing tests. We intend to examine this point in detail in the following report. p. 366

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At the close of this report I wish to thank the head and entire staff of the Kochi Prefectural Fisheries Station where the present study was conducted as part of tests aimed at eliminating damage due to disease, and where I was the object of much understanding and received much help.

I also thank the Shimahisa Yakuhin Co. for providing the ferment calcium lactate, and the Kōkin Kagaku Co. for providing the ethoxyquine.

Table 1. Effect of vitamin mixture of food used during experiment on the tolerance against streptococcicosis of yellowtail by the artificial peroral inoculation. The inoculum was injected under the skin of food fish

Food used during experiment		Exp-1a*	Exp-1b*	Exp-2a*	Exp-2b*	Exp-3	Exp-4	Exp-5
Without vitamin	Symptom positive (%)	67	67	30	33	70	38	53
	Pathogen positive (%)					0	6	47
	Mortality (%)	100	100	100	100	10	0	21
With vitamin mixture	Symptom positive (%)	40	50	50	50	87	24	41
	Pathogen positive (%)					7	0	47
	Mortality (%)	10	30	40	60	0	0	41

The foods used during experiment were thawed anchovy in Exp-1, thawed sand launce (small size) in Exp-2 thawed mackerel in Exp-3, thawed sardin in Exp-4, and deteriorated anchovy in Exp-5. The fishes examined were reared for 14 days in July in Exp-1, 10 days in September in Exp-2, 30 days in February in Exp-3, 30 days in March in Exp-4, and 30 days in November in Exp-5.

Strains used as the inoculum were St-34 in Exp-1, 2, 3, 4, and St-36 in Exp-5.

Dosed number of inoculum was 2×10^{10} cells per individual in Exp-1, 1×10^{10} in Exp-2, 8×10^{9} in Exp-3, 3×10^{9} in Exp-4, and 6×10^{9} in Exp-5.

* Experiments a and b were tried at the same time.

Table 2. Effect of vitamin and nutriments used during the experiment on the tolerance against streptococcicosis of yellowtail by the artificial peroral inoculation. The inoculum was injected under the skin of food fish

Food used experiment	Symptom positive		Pathogen positive		Mortality (%)
	Total (%)	Serious (%)*1	Total (%)	Serious (%)*2	
Without drags	83	42	75	67	100
With vitamines and ursodesoxycholic acid	100	33	67	42	100
With vitamines and garlic extract	85	27	18	9	100
With vitamines and calcium lactate	83	42	70	40	100

The food used during the experiments was thawed sardine. The period of experiment was 20 days.

The strain used as pathogen was St-36. Dosed number of inoculum was 9×10^{9} cells per individual.

*1 The symptoms were counted more than three. *2 The pathogen were counted more than 10^{4} CFU/g.

Table 3. Effect of food condition fed during experiment on the tolerance against streptococcicosis of yelowtail by the artificial peroral inoculation. The inoculum was injected under the skin of the food fish

Food used during experiment		Exp-1	Exp-2
Thawed	Symptom positive (%)	24	53
	Pathogen positive (%)	0	47
	Mortality (%)	0	41
Not-thawed	Symptom positive (%)	7	11
	Pathogen positive (%)	0	5
	Mortality (%)	0	0
Washed after thawing	Symptom positive (%)	—	21
	Pathogen positive (%)	—	31
	Mortality (%)	—	5
Thawed with ethoxyquine	Symptom positive (%)	—	37
	Pathogen positive (%)	—	26
	Mortality (%)	—	5

The food used during experiments were sardine in Exp-1 and anchovy in Exp-2. The period of experiment was 1 month in both cases. Strains used as pathogen were St-34 in Exp-1 and St-36 in Exp-2. Dosed number of inoculum was 3×10^9 cells per individual in Exp-1 and 6×10^9 in Exp-2.

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